

August 1, 1951.

Dr. M. R. Zelle,
Laboratory of Bacteriology,
Cornell University,
Ithaca, New York.

Dear Max:

Here, finally, are the summarized pedigrees on the single cell isolates of H-226. I will try to get out a similar summary on H-206, but the data on this are less extensive and less useful. Except that they show the generality of diploid behavior, the same holds for H-72 and H-168.

The most important point I noticed is that the segregation ratios of H-226 [M- Lac₄- // T-L- Lac₁- Mal- Xyl- Mtl-] may not be so discrepant after all. Random isolates, e.g., of Lac- from segregating cultures are very predominantly ~~xxx~~ Mal+ (86:4), but one can count segregant clones in these pedigrees as 7:10, or some similar figure not significantly different from 1:1. It must be concluded that selection plays a larger role than I had thought in the establishment of observed ratios. Picking individual Lac- from Lacy colonies does not eliminate selective differentials that may operate on ordinary complete medium without specific sugars, etc., and this seems to be the case here. Possibly some of the auxotroph markers ~~xxx~~ are involved; possibly quite cryptic differences.

I mentioned this detail in my CSH ms. as Zelle and Lederberg, 1951, and unpublished. I'm not sure whether I asked you specifically about this, and hope it is OK with you.

The families with segregants are in the small envelope; all other diploid pedigrees are included also. The interesting cases are: (-/+ refers to Mal)

2/5: D [15-22] -	2/18 G3 +	3/31 B61 +
G 103-110		F21 +
counted as - 3		A23-24 -
+ 1	2/24 B[28-30] -	
A 15 -	D 30 +	
E 107 -	G 16 -	6/5 H-12 +
110 -		
		(but from ^M Lac-Maly)

It is a little difficult to know how to count 2/5 G and 6/5 M, but the total was put down as 7+:10-. At any rate there certainly is no twenty-fold preponderance of Mal+ as there is in the "random" isolations.

The only other datum I've been able to extract from the pedigrees is the frequency of segregation. From 47 initial cells there were 654 diploids and 14 haploid clones (counting 2/5 G as one for this purpose). If the lethals (69 independent occurrences or clones) are assumed to occur at random, and can therefore be discounted, we can compute $654 + 14 - 47$ fissions at which segregation might occur, 14 at which it did, for a ratio of 2.3% or about 1:44. This is somewhat less, I believe, than the other diploids showed, but I'll have to go over them.

lethals.

I haven't tried to figure anything to do with the ~~lethals~~. They occur in pedigrees showing no haploid survivors, and also in a few pedigrees from haploid initials (not included in this bunch). I am a little doubtful now as to the value of ~~assessing~~ their occurrence as an explanation of aberrant ratios- it is quite possible that considerable "internal absorption", etc., occurs. The bunches of cells with unusual proportions of haploid progeny of certain types, but from which one could isolate typical diploids suggests a certain amount of persistent "mixoploidy", as of course the rather regular asymmetry of segregations does as well.

It would be very nice to have more data now on primary segregation ratios, but it is rather obviously not worth the work of more single-cell pedigrees. I'm trying to develop a substitute for this purpose, but letting small microcolonies start from single (?) cells inoculated with a pipette in a row on an agar plate. After a few hours, the microcolonies are strung out into rows of cells by a glass spreader stroked on the plate perpendicular to the original row. In reconstruction experiments it works rather well, but in practice on H-226 microcolonies I'm still having too much trouble with colonies from clumps including preexistent haploids. I may have to verify the single-cells in the initial rows ~~by~~ with the microscope, which begins to look like a poor compromise. If it can be made to work, I should be able to get the clones of haploids soon enough to mitigate selective forces.

Otherwise, we're in about the same status as before the GSH meetings. Esther and I are finishing a job using replica plating (velvet transfers) to clinch the proof for preadaptation. The replicas can be used, for example, to show that in films of growth on a plate, phage-resistant (or S^r) mutants occur in clones that show up as congruent resistants on a series of plates. We can also go back to the congruent site on the original plate, and by using such sites as inocula for new plates at higher dilutions, exercise an indirect selection for the mutants. After several enrichments of this sort, we have isolated the resistant mutants in pure culture from cells that have never been in contact with the selective agent- the selection being based entirely on the phenotype of the sibs of the indirect-selection line. This should turn out to be a more or less decisive method to settle the question one way or another in the various cases still floating around.

We drove home via Ithaca, and Ontario (Robinson), but unfortunately couldn't raise you on the phone the day we passed through. What's it like now to see your F-1 in front of you?

Sincerely,

Joshua Lederberg